reasoning from an earlier Office Action (mailed September 4, 1990) that:

> MBR state in a Smyczek communication of 7/13/87 that the MBR product was developed independent of the information provided by applicants. The communication further indicates (page 5) that using a detergent in the buffer is obvious since it was available because of previous use, and that it is not essential for stabilization.

See Office Action of September 4, 1990, page 5, as referenced in the Office Action of May 3, 1991, page 2.

The Examiner also notes that a Rule 131 declaration filed by the Applicants failed to overcome the anticipation rejection because:

(1) it was not signed by all of the inventors; and

(2) the evidence presented therein "fails to set forth that the nonionic polymeric detergent stabilizes the thermostable nucleic acid polymerase enzyme during storage".

See Office Action of May 3, 1991, page 2.

B. The New Rule 131 Declaration

The Applicants have now submitted a Rule 131 Declaration signed by all of the co-inventions of the claimed subject matter, namely: David H. Gelfand; Susanne Stoffel; and Randy Saiki. This declaration, made with the knowledge that willful false statements could result in fines, imprisonment, and/or invalidation of any patent issuing from the present application, unequivocally states that these

individuals invented the claimed subject matter of the present application prior to May 1, 1987.

Such a declaration should be accorded more evidentiary significance than the posturing of a party involved in a dispute which, at the time, appeared headed for litigation. The statements from the Smyczek communication (7/13/87) cited by the Examiner are nothing more than what would be expected from someone accused of breaching the confidentiality provisions of a materials transfer agreement.

C. The Examiner's Contentions Regarding MBR's Position

The Examiner's contention that the Smyczek communication "indicates (page 5) that using a detergent in the buffer is obvious since it was available because of previous use, and that it is not essential for stabilization" is not an accurate reflection of the content of that communication or the facts of the Cetus/MBR dispute. (See Office Action of September 4, 1990, page 5, as referenced in the Office Action of May 3, 1991, page 2). Nothing in the Smyczek communication suggests that MBR would have used a non-ionic detergent to stabilize a purified therostable nucleic acid polymerase composition without having first seen the Cetus protocol.

To the contrary, every step of MBR's decision-making process was dependent on information previously attributable to Cetus. First, and foremost, MBR never had a Taq DNA polymerase composition prior to receiving the Cetus protocol. Smyczek's communication does not refute this fact.

Also, Smyczek carefully words his discussion regarding the use of non-ionic detergents in a storage buffer as follows:

> a vast number of materials can be used to stabilize the enzyme, and in fact, various materials have been used in previous publications for that purpose, which include Bovine Serum Albumin and gelatin, to name just two.

See page 5 of the Smyczek communication. Despite the fact that the dispute between Cetus and MBR relates to the use of non-ionic detergents in the storage buffer, Smyczek completely avoids this issue, and does not even suggest, much less name, a single publication in which a non-ionic detergent is used to stabilize a thermostable nucleic acid polymerase composition.

Again, as stated by Smyczek himself, the reasons for choosing to use a non-ionic polymeric detergent in the storage buffer were dependent on prior information from Cetus rather than some knowledge already in MBR's possession. Specifically, MBR chose to use the non-ionic detergent, Tween 20, because:

1. MBR had a sufficient quantity of this detergent in stock due to its prior work with Cetus.

2. Since Cetus figured to be a logical customer for its enzyme, MBR employed a storage buffer which was compatible to Cetus' system. (Emphasis added.)

See page 5 of the Smyczek communication.

At no point in the Smyczek communication, does he ever state, suggest or even imply that the use of a

non-ionic polymeric detergent in the storage buffer for a thermostable nucleic acid polymerase composition was known or obvious to MBR. Smyczek simply avoids the issue because without the prior information from Cetus, MBR would never have used Tween 20 in its Taq DNA polymerase composition.

Further evidence of the novelty and non-obviousness of using non-ionic polymeric detergents in a thermostable nucleic acid polymerase storage buffer can be found in MBR's own product literature. Prior to its release of a Taq DNA polymerase in 1987 (the product literature of which has been cited by the Examiner), MBR marketed three DNA polymerases:

(1) E. coli DNA Polymerase I;
(2) E. coli DNA Polymerase I, Klenow Fragment; and
(3) T4 - Infected E. coli DNA Polymerase.

See MBR Product List of December 8, 1986 and product literature for each DNA polymerase, attached as Exhibit A. However, not one of the storage buffers for these MBR polymerases contained a non-ionic polymeric detergent. See Exhibit A.

Examination of the MBR product literature shows that the DNA polymerase storage buffers used by MBR prior to its receipt of the Cetus protocol bear little resemblance to the storage buffer for MBR's Taq DNA polymerase. MBR's storage buffers for E. coli DNA Polymerase I, E. coli DNA Polymerase I, Klenow Fragment and T4-Infected E. coli DNA Polymerase contain potassium phosphate, dithiothreitol and glycerol, whereas the MBR Taq DNA polymerase storage buffer (only

marketed after receipt of the Cetus protocol) contains TrisHCl potassium chloride, EDTA, dithiothreitol, Tween 20 and glycerol.

In view of the candid disclosure by Cetus of the correspondence between itself and MBR, the carefully worded statements in the letters from MBR, and the declaration (under possible penalty of patent invalidation) by the inventors of the claimed subject matter of the present application, it is respectfully suggested that the Applicants' version of the facts as presented in the declaration clearly establish their prior invention of the claimed subject matter.

D. The Evidence of Prior Invention

The declaration of the inventors is further supported by the attachments thereto, particularly the Taq polymerase purification protocol sent to James F. Wick of MBR. This protocol specifically states that the resultant enzyme composition (a purified thermostable nucleic acid polymerase enzyme) is then stored in a 2 X storage buffer. Two components of this storage buffer are identified as Tween 20 and NP-40 (non-ionic polymeric detergents).

It is only logical that this resultant enzyme composition is a stable enzyme composition, otherwise the composition would not be capable of storage. Stated another way, there is no reason to store an unstable enzyme composition. If the resultant enzyme composition from the Taq polymerase purification protocol were unstable, then the protocol would have indicated a need to use that enzyme composition immediately or within some limited time period rather

than stating that the enzyme composition could be stored.

Thus, what is described as the resultant product of the Taq polymerase purification protocol is exactly what is claimed in claim 1 of the present application:

> 1. A stable enzyme composition comprising a purified thermostable nucleic acid polymerase enzyme in a buffer and further comprising one or more non-ionic polymeric detergents.

Rule 131 (37 C.F.R. § 1.131) requires that any declaration filed pursuant thereto present "facts showing a completion of the invention in this country before the . . . date of the printed publications." As explained in greater detail above, the Rule 131 declaration filed by the Applicants clearly present(s) facts showing that before the date of the MBR product information sheet, these Applicants had completed the invention of:

> (1) a stable enzyme composition (the resultant product of the Taq polymerase purification protocol did not have to be used right away; it could be stored, thus indicating its stability);
>
> (2) comprising a purified thermostable nucleic acid polymerase enzyme in a buffer (comprising is synonymous with including, and the resultant product of the Taq polymerase purification protocol included purified Thermus aquaticus (thermostable) DNA (nucleic acid) polymerase enzyme in a 2X storage buffer);

(3) and further comprising one or more non-ionic polymeric detergents (the resultant product of the Taq polymerase purification protocol also included Tween 20 and NP-40, non-ionic polymeric detergents).

Therefore, the Rule 131 declaration filed by the Applicants clearly meets the requirements of that Rule, and thus, establishes completion of the claimed invention in this country before the date of publication of the MBR product specification sheet.

In discussing the alleged inadequacy of the previously submitted Rule 131 declaration, it was also asserted that:

> the MBR buffer is a species different from the 2X buffer and both are within the scope of the present claims. In chemical cases, where generic claims have been rejected on a reference which discloses a species not antedated by the affidavit or declaration, the rejection is not withdrawn (MPEP 715.03).

See Office Action mailed May 5, 1991, page 3. This differentiation of species was alleged because "[f]or example, the 2X buffer contains NP40 and Tween 20 whereas the MBR buffer contains only Tween 20, and the proportions of all components of the MBR buffer are different from the proportions of the components of the 2X buffer." Id. Specifically, the proportions of the components of the Cetus 2X storage buffer are exactly twice the proportions of the components of the MBR buffer. Compare Exhibit B (MBR product literature) and Exhibit C (last page of Cetus protocol describing the 2X buffer).

However, the Examiner fails to take account of a final dilution step in the Cetus protocol which renders the proportions of all components of the MBR buffer virtually identical to the proportions of the respective components of the 2X storage buffer. Specifically, the last sentence of the Cetus protocol requires the addition of an "equal volume of 90% glycerol . . . ." The addition of an equal volume of glycerol results in a 50% dilution of the 2X buffer. Thus, both the MBR buffer and the 2X storage buffer from the Cetus protocol are of the same species because both contain 20 mM Tris-Cl, 0.1 M KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Tween 20 and about 50% glycerol (the Cetus storage buffer actually contains 47.5% glycerol which serves the same purpose as 50% glycerol, that is to prevent the enzyme composition from freezing at -20°C).

Furthermore, even if the MBR buffer and the 2X storage buffer were not identical, the Examiner's definition of species is too narrow for evaluation under the provisions of MPEP § 715.03. Specifically, this section of the Manual of Patent Examining Procedure relates to ". . . chemical cases, where generic claims have been rejected on a reference which discloses a species not antedated by the affidavit or declaration. . . ." In the present application, the generic aspect of the claim is "non-ionic polymeric detergents," and the rejection is based on a reference which discloses a species of non-ionic polymeric detergents, namely Tween 20.

Therefore, the Applicants' Rule 131 declaration and supporting documents which establish the prior invention of a stable thermostable enzyme composition

including Tween 20 show the same species of non-ionic polymeric detergent as described in the MBR product specification sheet. Thus, MPEP § 715.03 is not applicable because the MBR species of non-ionic polymeric detergent (Tween 20) is antedated by the declarations which show the prior invention with the same species of non-ionic polymeric detergent, Tween 20.

Alternatively, even if the Examiner is not persuaded that the Applicants invented the claimed species of the invention prior to the publication of the same species by MBR, the Applicants have also satisfied the mandates of MPEP § 715.03 which require that the Applicants establish possession of the generic invention prior to the effective date of the reference. As explained above, the Taq polymerase purification protocol provided to MBR before May 1, 1987, fully described how to make and use a stable enzyme composition as set forth in claim 1.

E. Conclusion Regarding Anticipation

Based on the Rule 131 Declaration, the accompanying documents, and a reasonable review of the correspondence between Cetus and MBR, it is believed that the anticipation rejection has been overcome, and withdrawal of the rejection is respectfully requested.

II. Obviousness (35 U.S.C. § 103)

Numerous obviousness rejections have been raised, each of which is addressed individually below.

A. Claims 40, 41 and 60-62

Claims 40, 41 and 60-62 stand rejected as obvious over the MBR product information sheet because:

> [i]t would have been a matter of obvious choice and depend on individual preference and convenience to vary somewhat the pH and/or concentration of the buffer composition of MBR and/or use a species of Thermus other than aquaticus disclosed by MBR know to produce polymerase as a source of polymerase. Using the polymerase product of MBR in a reaction mixture as required by claim 62 would have been obvious since polymerase is normally reacted in such a mixture.

See Office Action of September 4, 1990, page 6, as referenced in the Office Action of May 3, 1991, page 3. The Examiner then went on to assert that the Rule 131 affidavit failed to eliminate the MBR reference. See Office Action of May 3, 1991, page 3.

As with the anticipation rejection discussed above, the Applicants respectfully submit that the Rule 131 Declaration filed herewith clearly establishes that the Applicants invented the claimed subject matter of the present application prior to the publication date of the MBR reference. Thus, the MBR reference is not prior art to the present application and it is respectfully requested that this obviousness rejection be withdrawn.

B. Claims 1, 35-41, 53-59 and 62

1. The Rejection

Claims 1, 35-41, 53-59 and 62 stand rejected as obvious over Kaledin et al. (1980) in view of Goff et al. and, if necessary, in further view of Feller et al. or Spiegelman as:

> [i]t would have been obvious to store the polymerase of Kaledin et al. in a buffer containing a nonionic detergent in view of Goff et al. disclosing (col. 8, line 24) that a nonionic detergent is required in recovering this enzyme and if needed in further view of Feller et al. (col. 5, line 7) or Spiegelman (col. 6, line 25) disclosing use of a detergent-containing buffer in relation to this type of enzyme.

See Office Action of September 4, 1990, page 6, as referenced in the Office Action of May 3, 1991, page 4. The Examiner then went on to assert that although Kaledin et al. teach the use of gelatin to stabilize their enzyme,

> when Goff et al. and, if needed, Feller et al. or Spiegelman are considered, it would have been apparent that in addition to gelatin a nonionic detergent would have also functioned to stabilize the polymerase. To use an alternative stablizing agent known for polymerase stabilization would have been a matter of obvious choice depending on individual preference and convenience. Furthermore, to select a preferred stabilizing agent from these known would have required only limited routine experimentation and have been within the skill of the art.

See Office Action of May 3, 1991, page 4.

2. The Rule 132 Declarations Refute the Rejection

The assertions regarding obviousness of a choice between gelatin and non-ionic detergent as a stabilizer for a thermostable nucleic acid polymerase based on the disclosure of Goff et al. is refuted by the evidence presented in the accompanying Rule 132 declarations of James Akers and David H. Gelfand.

a. The Akers Declaration

Briefly, Mr. Akers performed a series of experiments to address the following issues:

(1) whether the presence or absence of non-ionic detergent in a reverse transcriptase (RT) storage buffer has an equivalent effect on the functionality of RT;

(2) whether the absence of non-ionic detergent in a thermostable nucleic acid polymerase storage buffer effects the functionality of a thermostable nucleic acid polymerase; and

(3) given the requirement for a non-ionic detergent in a thermostable nucleic acid polymerase storage buffer to maintain functionality, whether gelatin is equivalent to non-ionic detergent.

Mr. Akers' tests show that:

(1) RT is not effected by the presence or absence of non-ionic detergent;

(2) the functionality of Taq DNA polymerase (a representative thermostable nucleic acid polymerase) is severely diminished in the absence of non-ionic polymeric detergent; and

(3) gelatin is not equivalent to non-ionic polymeric detergent for effecting the activity of Taq DNA polymerase.

Specifically, as set forth in his declaration, Mr. Akers performed an experiment (Experiment No. 1) in which the functional activity of a pure RT was compared when that enzyme was stored with and without the non-ionic polymeric detergent, NP-40. The results of this experiment showed that RT functions equivalently in an RNA-PCR protocol whether stored in a buffer with NP-40 or without NP-40.

In Experiment No. 2, Mr. Akers used the same RNA-PCR protocol to compare the functional activity of Taq DNA polymerase when stored with and without the non-ionic polymeric detergents, NP-40 and Tween 20. The results of this experiment indicate that there is a minimal amount of non-ionic polymeric detergent necessary for the functional activity of Taq DNA polymerase.

Finally, in Experiment No. 3, Mr. Akers again used the standard RNA-PCR protocol to compare the functional activity of a Taq DNA polymerase composition with NP-40 and Tween 20 to the functional activity of the Taq DNA polymerase composition in which NP-40 and Tween 20 were replaced by gelatin. Again, the results of this

experiment confirmed that some minimal amount of non-ionic polymeric detergent is necessary for the functional activity of Taq DNA polymerase; gelatin alone does not maintain the functional activity of Taq DNA polymerase.

b. The Gelfand Declaration

In addition to Mr. Akers' experiments which unequivocally demonstrate that the effects of a non-ionic polymeric detergent on a thermostable nucleic acid polymerase are not predictable from its effects on RT, Dr. Gelfand has also presented evidence in his declaration that further substantiates the unpredictability of thermostable nucleic acid polymerase properties from RT properties. Briefly, Dr. Gelfand utilized a computer program which identifies similarities in amino acid sequences. Whereas, there was no meaningful similarity between the amino acid sequences of murine leukemia virus reverse transcriptase (MuLV RT) (Goff et al.) and Taq DNA polymerase, there was a much higher degree of similarity between the amino acid sequences of Taq DNA polymerase and E. coli DNA polymerase I.

Dr. Gelfand also explained that the significantly different isoelectric points of MuLV RT and Taq DNA polymerase are further evidence of these enzymes' dissimilarities, and reflect the significant differences in their respective amino acid compositions. The significantly different isoelectric points of these two enzymes indicate that they will exhibit significantly different migration patterns during isoelectric focussing analytical procedures.

From the information presented in his declaration, Dr. Gelfand concluded that someone of ordinary skill in the art would not expect the properties and behavior of MuLV RT to be predictive of the properties and behavior of a thermostable nucleic acid polymerase.

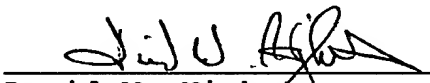
3. Conclusion Regarding Obviousness

Thus, it is respectfully submitted that the references cited by the Examiner (Goff et al., Feller et al. and Spiegelman, all of which relate to the use of detergents with RT) do not provide sufficient teaching such that one of ordinary skill in the art would find the claimed invention of the present application to be obvious. Therefore, withdrawal of the obviousness rejections of the pending claims is respectfully requested.

III. Conclusion

In view of the above remarks and the evidence presented in the accompanying declarations, the allowance of the claims of the present application is proper. The prompt issuance of a Notice of Allowance is earnestly requested.

Respectfully submitted,



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